

## Research Paper

## Temporal photoperiod sensitivity and forcing requirements for budburst in temperate tree seedlings

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## ABSTRACT

Phenological responses to winter and spring warming in trees alter growing season length and can influence productivity. An improved mechanistic understanding of phenology, including temporal changes in budburst forcing requirements (BFR) and photoperiod sensitivity, could improve projections of phenological shifts and changes in tree species composition in response to climate warming. We investigated changes in BFR and photoperiod sensitivities at high temporal resolution from mid-winter to spring in seedlings of eight common deciduous and coniferous temperate tree species. Eight provenances of *F. sylvatica*, a dominant European species, also were included to examine variability in bud dormancy patterns within a species. Tree seedlings were overwintered in a common garden and transferred weekly into climate chambers at forcing temperatures (+20 °C) from December to April. Budburst was observed under 16 and 8 h photoperiods. Across species, as chilling unit sums accumulated, BFR and photoperiod sensitivity decreased. Functions relating chilling and forcing unit sums explained ambient spring budburst accurately. BFR differed strongly among species, but not among provenances of *F. sylvatica* from similar latitudes. Overall, our results indicate that a precise tracking of BFR and photoperiod sensitivity helps explain species-specific differences in phenotypic sensitivities, which can improve species-specific projections of phenological responses to climate warming.

## 1. Introduction

Changes to the start of the growing season in trees impact primary productivity (Richardson et al., 2010) and can have wide reaching implications for plant-animal interactions due to potential changes in the timings of foraging and pollination (Both et al., 2009). In Europe, the growing season advanced on average by 11 days from the 1960s to the 21st century, mostly due to earlier leaf emergence (Linderholm, 2006; Menzel et al., 2006). The timing of spring growth plays a vital role in influencing biomass production by modifying the growing period, with budburst dates influencing carbon assimilation and tree energy budgets (Kindermann et al., 1996). For example, a 20% extension in the growing season can increase the annual net ecosystem productivity of a deciduous forest by as much as 50% (Dragoni et al., 2011), although carbon sequestration may not necessarily increase due to higher respiration rates (Piao et al., 2008). Nevertheless, the tradeoff of earlier leaf flushing is an increased risk of late spring frost damage

(Gömöry and Paule, 2011; Mimura and Aitken, 2010).

Evidence shows that budburst can be altered by modifying the temperature (Fu et al., 2012) and photoperiod (Myking and Heide 1995) after bud set; therefore, abiotic environmental changes likely drive variability in spring budburst dates. Observed advancements in spring phenology have been species-specific (Cleland et al., 2007; Laube et al., 2014; Menzel et al., 2006; Willis et al., 2008), largely due to species-specific requirements for the breaking of bud dormancy. Temperature and photoperiod are the most important factors controlling phenology in dominant tree species outside the tropics (Körner, 2007).

Bud phenology is driven mechanistically by changes in (or the absence of) bud dormancy. In many temperate deciduous trees, endodormancy or “the inability of a bud to burst at normal growth temperatures in long days” (Sogaard et al., 2008), is released by a required chilling period (Laube et al., 2014). Once endodormancy is completely released, trees enter eco-dormancy, where the buds become

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**Table 1**

Geographical and climatic characteristics of the seed origins of the tree seedlings used in the experiment, and the respective tree seedling ages at the start of experiment.

Species	Latitude °N	Longitude °E	Elevation	Sowing date	age (years)
<i>Acer pseudoplatanus</i> L.	49.89	11.05	409–537	2012 (April)	2
<i>Picea abies</i> L.	49.90	10.50	445–450	2010 (May)	4
<i>Fagus sylvatica</i> L.	50.04	11.85	800–920	2013 (May)	1
<i>Abies alba</i> Mill.	49.96	11.04	325–379	2009 (October)	4
<i>Sorbus torminalis</i> L.	49.84	10.38	270	2011 (June)	2
<i>Larix decidua</i> Mill.	50.08	9.25	440	2012 (May)	2
<i>Tilia cordata</i> Mill.	49.45	11.14	330	2011 (June)	2
<i>Quercus robur</i> L.	49.52	11.06	307–311	2013 (May)	1

increasingly responsive to warm forcing temperatures (Harrington and Gould, 2015; Kramer, 1994). Short photoperiods can prevent premature dormancy release when the chance of frost still may be high (Häkkinen et al., 1998; Heide, 1993a), while long photoperiods can compensate for insufficient winter chilling temperatures by reducing budburst sensitivity to warmer temperatures (Häkkinen et al., 1998; Heide, 1993b; Sanz-Pérez et al., 2009). Budburst sensitivity to photoperiod is of particular importance for assessing tree growth responses to climate warming (Heide, 1993b; Schaber and Badeck, 2003; Vitasse et al., 2009), because photoperiod plays a role in bud dormancy release and budburst (Basler and Körner, 2014; Partanen et al., 1998; Zohner and Renner, 2015), and can limit the sensitivities of some tree species to warmer winter and spring temperatures (Way and Montgomery, 2014).

The interplay between chilling requirements, forcing temperatures, and photoperiod in influencing budburst is species-specific (Vitasse et al., 2009); longer photoperiod and longer exposure to chilling temperatures reduce the thermal time to budburst in some species (Falusi and Calamassi, 1990; Heide, 1993b), but have no effect in others (Heide, 1993b; Schaber and Badeck, 2003). Required chilling temperature sums differ among Northern species (Farmer, 1968). For example, *Picea abies* requires four weeks of chilling at 3–6 °C to break dormancy (Dormling et al., 1968), whereas *Pinus monticola* requires 16 weeks of chilling at the same temperature (Steinhoff and Hoff, 1972). Tree species that advance their budburst dates the most typically have low forcing and chilling temperature requirements for budburst (Laube et al., 2014; Zohner and Renner, 2014).

Regarding changes in BFR and photoperiod sensitivity from winter to spring, photoperiod sensitivity has been shown to decrease in selected species as chilling requirements are fulfilled (Caffarra and Donnelly, 2011; Laube et al., 2014; Myking and Heide, 1995). With respect to changes in BFR, several studies have shown that the relationship between the accumulated chilling unit sum and BFR may be exponential, with BFR decreasing exponentially from midwinter to spring (Caffarra and Donnelly, 2011; Harrington et al., 2010; Heide, 1993b; Murray et al., 2014; Myking and Heide, 1995). Forcing requirements decrease as chilling units accumulate, until a point where they plateau and remain stable with additional chilling unit accumulation (Harrington et al., 2010; Harrington and Gould, 2015). When budburst dates have been analyzed in the context of hourly chilling and forcing sums, the required amount of forcing units needed for budburst could be explained by the specific amount of chilling units that had accumulated up to that point (Ford et al., 2016; Harrington et al., 2010). However, in order to test the generality of these relationships between exponential chilling unit sums vs. BFR, and the continuous decrease in photoperiod sensitivity, these need to be evaluated for multiple species at a fine resolution.

Phenological differences among tree populations from different longitudes, latitudes and elevations within single species also have been documented (Von Wuehlisch et al., 1995; Chmura and Rozkowski, 2002), and these within-species differences in bud phenology can at least in part be explained by genotypic variation (Campbell et al., 1989; Ekberg et al., 1991). Although environmental effects appear to explain much more variation in budburst dates than genetic differences (Vitasse

et al., 2013), genetic control, which is observed down to the seed family level, enables budburst order in seedlings to be maintained across years with varying temperature (Li and Adams, 1993). Therefore, within-species variation in bud phenology should be considered whenever phenology is compared among species.

We used days to budburst under forcing conditions, a proxy for dormancy level (Li et al., 2005) to quantify changes in BFR from mid-winter to spring in eight common European tree species known to differ in spring budburst dates (Kramer, 1995). Temporal changes in BFR and photoperiod sensitivity were then used to explain the spring budburst of each species in the field. We also explored variation in BFR within *Fagus sylvatica*, one of the most photoperiod sensitive (Kramer, 1994) and dominant tree species in Europe (Vitasse and Basler, 2013). We hypothesized that: 1) functions correlating changes in BFR with chilling unit sums would be exponential across species and would accurately predict the BFR at spring budburst, 2) higher mid-winter BFR and a slower rate of decrease in BFR would lead to later spring budburst dates both among species and among *F. sylvatica* provenances (due to adaptation to variation in climate at seed origin) and 3) photoperiod sensitivity would decrease continuously with chilling unit accumulation, and would differ more strongly among species than among *F. sylvatica* provenances (due to similar latitude at seed origin).

## 2. Materials and methods

Seven species of tree seedlings (*Abies alba* Mill., *Picea abies* L. H. Karst., *Quercus robur* L., *Acer pseudoplatanus* L., *Sorbus torminalis* L. Crantz, *Tilia cordata* Mill., *Larix decidua* Mill.) obtained from local German seed sources (Table 1) were grown in a tree nursery in the vicinity of Bayreuth, Germany (Bayerische Staatsforsten AG – Pflanzgarten-Stützpunkt Bindlach), and delivered to the Ecological Botanical Garden in Bayreuth at the end of October 2013. Seedlings from all provenances and tree species were transplanted into 8 cm × 8 cm × 20 cm deep pots at the end of October using soil from the Ah and Bv horizons of a forest soil in Eberswalde, Germany, made up predominantly of 87.2% sand, 9.8% silt, 2.9% clay and < 0.5% humus.

We selected eight *F. sylvatica* provenances from seed sources in northern France, northern Germany and Poland to represent the potential variation in chilling requirements (due to differences in winter climate at their seed origins), while likely retaining similar photoperiod sensitivities among provenances (due to the seed origins stemming from similar latitudes (Table 2). The trees were cultivated from seed in greenhouses at the Thünen-Institute, Germany (Institute of Forest Genetics, Institute of Forest Ecosystems), with the exception of one provenance (Table 2 – Germany 3), which was cultivated together with the other tree species as described in the preceding paragraph. In late fall 2012, the two-year old seedlings were potted in 2 L pots (Hermann Meyer KG), and in the summer of 2013 they were delivered to the Bayreuth Ecological Botanical Garden and placed under a rainout shelter constructed of a steel frame (GlasMetall Riemer GmbH) and covered with a polyethylene sheet (0.2 mm, SPR5, Hermann Meyer GmbH), which permitted 90% of photosynthetic radiation to pass

**Table 2**

Geographical and climatic characteristics of the seed origins of the *Fagus sylvatica* L. provenances used in the experiment, representing the within-species diversity category. Climate data were obtained from worldclim (Hijmans et al., 2005), using a resolution of 10 arc-seconds. The provenance “Germany 3” was used in the among-species comparison, because it had a similar seed origin and experienced the same growing conditions as the other species in the experiment.

Country	Latitude °N	Longitude °E	Altitude (m) a.s.l.	Mean minimum temperature of coldest month (°C)	Mean maximum temperature of warmest month (°C)	Annual mean temperature (°C)
France	50.25	1.88	< 200	1.2	21.6	10.3
Germany 1	52.95	8.35	< 200	− 2.3	21.4	9.0
Germany 2	53.40	9.83	< 200	− 3.2	22.0	9.2
Germany 3	50.04	11.85	800–920	− 5.7	19.8	5.9
Poland 1	52.68	17.67	< 200	− 6.2	23.5	8.2
Poland 2	53.08	18.93	< 200	− 6.3	23.6	7.8
Poland 3	53.27	19.50	< 200	− 7.2	23.3	7.8
Poland 4	53.02	19.60	< 200	− 7.7	23.5	7.8

through. The seedlings were kept under the shelters until fall and received one liter of water per week per pot. The same substrate was used as for the other species.

### 2.1. Overwintering conditions and treatments

Potted seedlings ( $n = 34\text{--}36$  per species and per provenance) were buried in a sand bed to the brims of the pots in the Ecological Botanical Garden of the University of Bayreuth, where they overwintered until spring 2014. Outside ambient temperature at the overwintering site was recorded at plant height ( $\sim 25$  cm) with temperature loggers (HOBO Pro v2, Onset Computer Corporation, Massachusetts USA),

Starting on 13 December, every 7–10 days, 2 seedlings from each provenance and species were transferred from the sand bed to climate chambers (Fig. 1). One seedling was kept at a long photoperiod (16 h) and one at a short photoperiod (8 h), both at 16 °C and 20 °C nighttime and daytime temperatures, respectively. It should be noted that more natural light regimes, simulating continuous increases day length, could lead to different results, as found for *P. abies*, which Partanen et al. (1998) observed was light-sensitive under progressively increasing and decreasing photoperiods. Mean humidity was maintained at around 50% ( $\pm 10\%$  st. dev.)

Every two days the plants were watered with approximately 50 mL of deionized water per pot and budburst was defined as the first green foliage visible on any one bud. Due to the different nature of budburst among the tested species (several buds always burst almost simultaneously for *L. decidua*, whereas for *S. torminalis*, distinct terminal buds

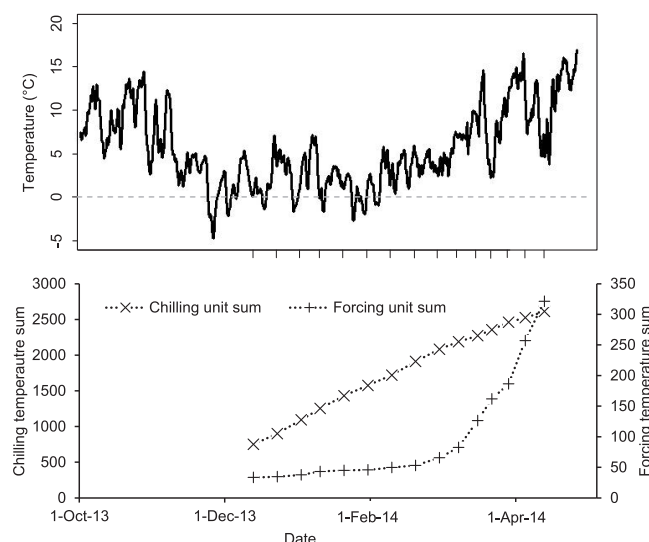
always burst first), no distinction was made between terminal and lateral budburst. In many instances, terminal and lateral budburst dates differed by less than 3 days, which made the distinction difficult. Still, predominantly terminal budburst was observed and recorded. Seedling locations, along with the temperature and photoperiod settings, were alternated every four days between the two chambers to minimize potential chamber-specific effects. Mean temperature for the duration of the experiment differed less than 0.5 °C between the long and short photoperiod treatments. In addition to recording budburst dates inside the chambers, ambient budburst was recorded in the field in the spring for seedlings of each species and provenance ( $n = 4\text{--}6$  per species and provenance). Ambient budburst and ambient budburst forcing requirements therefore refer to tree seedlings that had experienced no artificial warming and that leafed out under ambient field conditions. “Sampling dates” refer to the dates when the tree seedlings were moved into the growing chambers.

In order to characterize changes in environmental factors over time at a sufficient resolution, high sampling frequency in combination with low replication per sampling interval has been recommended, as opposed to using high replication for few sampling intervals (Schweiger et al., 2015). We therefore used one tree seedling per sampling date per photoperiod treatment (i.e. we allocated the 15 tree seedlings per species across the maximum number of sampling dates), in order to best characterize the function describing BFR changes. The  $R^2$  values of the functions, and the ability of the resulting functions to describe ambient budburst accurately for most species, were used retroactively to judge whether the treatment effects were large enough to outweigh any potential variability within sampling dates. The following observations provided support for the effectiveness of our approach:

- 1) The coefficients of determination between sampling dates and days to budburst that we obtained were as high as those reported in the literature (Table 3; Murray et al., 1989). The latter was expected because we simply exchanged high replication within time points with more frequent sampling in time (see above).
- 2) Spring budburst in the field was well-explained by the fitted functions. Accurate projections could be made for the ambient forcing units that had accumulated by the spring budburst date in the field (Table 3). That is, all eight species-specific ambient spring budburst dates could be explained by the correlations between the chilling unit and forcing unit sums.
- 3) Taking the mean of the responses of the eight different ecotypes of *F. sylvatica* at each sampling point had neither an appreciable effect on the coefficients of determination (Fig. 3), nor on the projected budburst dates (and therefore on the pattern of loss of BFR).

### 2.2. Calculation of chilling and forcing units

We calculated the accumulated hourly temperature sums in the chilling range, along with the hourly forcing temperature sums, from 1 November up to each sampling date (Fig. 1). November 1 was chosen because few chilling units accumulate before November in the region



**Fig. 1.** Top panel: air temperature (at 25 cm height) at the field site of the overwintering tree seedlings (24 h running mean shown). Bottom panel: chilling and forcing unit sum accumulation up to each of the 15 sampling dates.

**Table 3**

Significance and coefficients of determination for the non-linear correlations of sampling dates with days to budburst (top panel) and chilling unit sums with forcing unit sums (bottom panel), at long (16 h) and short (8 h) photoperiods, respectively. Significant photoperiod effects represent a depression of the days to budburst/forcing sum requirements at the longer photoperiod. Significant photoperiod interactions represent the influence of sampling date/chilling unit accumulation on the power of photoperiod to change budburst forcing requirements. Projected budburst dates/forcing unit sums show how accurately both regression types (sampling date vs. days to budburst, and chilling unit sum vs. forcing unit sum) could explain the respective ambient spring budburst dates/forcing units in the field. Bold numbers show significant effects at  $\alpha = 0.05$  and correctly projected budburst dates/required forcing units. Polynomial correlations were fitted as:  $y = ax^2 + bx + c$ , where  $x$  was a sampling date or a chilling sum, and  $y$  was the number of days to budburst or the forcing sum. Exponential correlations were fitted as:  $y = a * e^{(-b^x)}$ , where  $x$  was the chilling unit sum and  $y$  was the forcing unit sum. The constants of the equations are displayed in Table 1 of the supplemental information.

Sampling date vs days to bud burst fitted functions									
Species	Regression type	At long photoperiod		At short photoperiod		Photoperiod effect (mean effect size)		Photoperiod interaction	
		p	R <sup>2</sup>	p	R <sup>2</sup>	p		p	
								Projected budburst range (days since Dec 1)	Ambient day of budburst (+/- standard error)
<i>F. sylvatica</i>	Polynomial	< 0.01	0.91	< 0.01	0.95	< 0.01		151–155	143 +/- 1
<i>A. alba</i>		< 0.01	0.88	< 0.01	0.98	< 0.01		121–127	125 +/- 2
<i>S. torminalis</i>		< 0.01	0.67	< 0.01	0.94	0.02	0.09	109–121	116 +/- 4
<i>L. decidua</i>		< 0.01	0.95	< 0.01	0.88	0.07	0.29	111–118	104 +/- 1
<i>T. cordata</i>		< 0.01	0.90	< 0.01	0.94	< 0.01	0.02	138–162	146 +/- 1
<i>A. pseudoplatanus</i>		< 0.01	0.92	< 0.01	0.94	0.38	0.83	145–148	135 +/- 6
<i>Q. robur</i>		< 0.01	0.92	< 0.01	0.99	0.23	0.60	131–131	134 +/- 7
<i>P. abies</i>		< 0.01	0.80	< 0.01	0.81	0.48	0.49	147–148	146 +/- 2

Chilling unit sum vs. forcing unit sum fitted functions									
Species	Regression type	p		p		p		p	
			R <sup>2</sup>		R <sup>2</sup>				
								Projected forcing unit sum range	Ambient forcing temperature unit sums at budburst
<i>F. sylvatica</i>	Exponential	< 0.01	0.86	< 0.01	0.92	< 0.01	(< 0.01)	498–380	386 +/- 20
<i>A. alba</i>		< 0.01	0.81	< 0.01	0.97	< 0.01	(< 0.01)	256–234	235 +/- 40
<i>S. torminalis</i>		0.05	0.53	< 0.01	0.87	0.02	(0.07)	174–161	175 +/- 80
<i>L. decidua</i>		< 0.01	0.94	< 0.01	0.80	0.10	0.29	139–118	110 +/- 20
<i>T. cordata</i>	Polynomial	< 0.01	0.73	< 0.01	0.88	< 0.01	(< 0.01)	351–413	415 +/- 20
<i>A. pseudoplatanus</i>		< 0.01	0.88	< 0.01	0.90	0.40	0.84	377–306	314 +/- 120
<i>Q. robur</i>		< 0.01	0.82	< 0.01	0.97	0.18	0.51	240–245	301 +/- 140
<i>P. abies</i>		0.13	0.30	0.02	0.41	0.33	0.37	292–403	410 +/- 40

and the same date had been used in previous studies (Basler and Körner, 2012; Murray et al., 2014; Zohner and Renner, 2015). The exact values needed to attain the forcing and chilling units were calculated for each hour using the following equations (thought to apply across many species) presented by Harrington et al. (2010):

$$CU = 3.13 \left( \frac{T + 4.66}{10.93} \right)^{2.10} e^{-\left( \frac{T + 4.66}{10.93} \right)^{3.10}} \quad (1)$$

$$FU = \frac{1}{1 + e^{-0.47T + 6.49}} \quad (2)$$

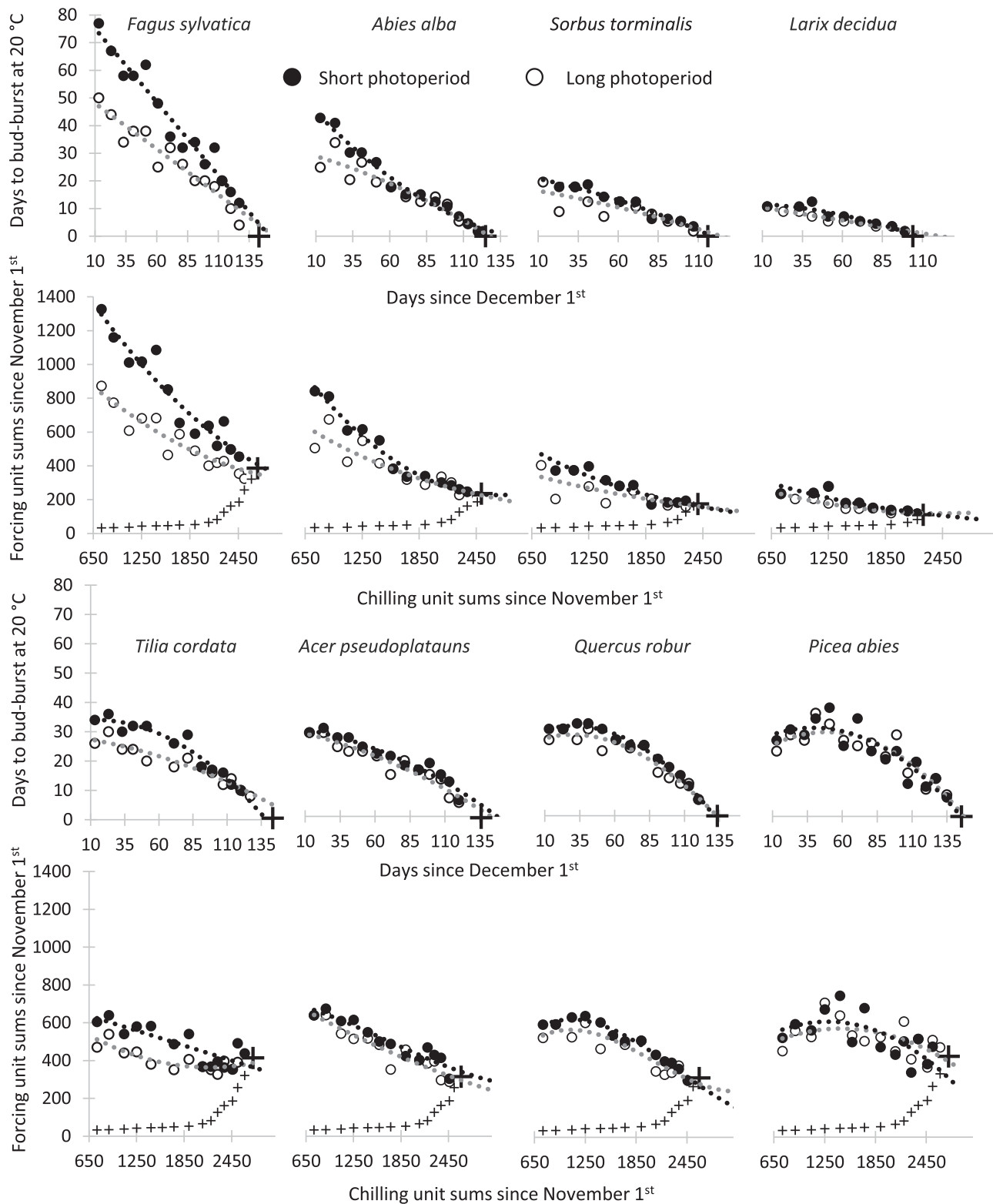
These functions have been used successfully by previous studies to test the effectiveness of each temperature for the accumulation of chilling and forcing units (Harrington et al., 2010; Harrington and Gould, 2015). CU represents chilling units and FU represents forcing units.  $T$  is the hourly temperature and  $e$  is the base of the natural logarithm. CU is set to zero when  $T < -4.7^\circ\text{C}$ , or  $T > 16^\circ\text{C}$ , and set to 1.0 when Eq. (1) yields a value  $> 1.0$ .

We first plotted the days to budburst in the climate chambers for each sampling date (presented as days since 1 December; top panels of Fig. 2). For the correlations of chilling unit sum vs. forcing unit sum, we plotted the chilling and forcing unit sums which had accumulated up to each sampling point (at the field site), plus the forcing units which had accumulated in the climate chambers (calculated using Eqs. (1) and (2), respectively; bottom panels of Fig. 2). Also, as suggested by Harrington et al. (2010), both chilling and forcing units were thought to influence budburst throughout winter (as opposed to sequential models), so both sums were calculated from 1 November (a common start point when bud formation is complete).

### 3. Statistical analyses

#### 3.1. Effect of sampling date/chilling unit sums on BFR

Changes in BFR for each photoperiod were fitted to functions using (1) the raw data (with sampling date as the predictor variable, and days to budburst in the climate chambers as the response variable) and (2) calculated chilling and forcing temperature sums for each sampling date (with chilling unit sum as the predictor variable and forcing unit sum as the response variable). Polynomial functions were fitted for the correlations of sampling date vs. days to budburst;  $y = ax^2 + bx + c$ , where  $x$  was the sampling date, and  $y$  was the days to budburst in the climate chamber (fitted to best explain the variance in the data while minimizing the number of unknowns). Exponential or polynomial functions were fitted for the correlations of chilling unit vs. forcing unit (polynomial functions resulted in a much better fit for *Quercus robur* and *Picea abies*; Fig. 2). Exponential correlations were fitted as:  $y = a * e^{(-b^x)}$ , where  $x$  was the chilling unit sum and  $y$  was the forcing unit sum ( $a$  and  $b$  were constants). Polynomial correlations were fitted as:  $y = ax^2 + bx + c$ , where  $x$  was the chilling unit sum and  $y$  was the forcing unit sum. The choice between fitting polynomial vs. exponential functions to correlate chilling and forcing unit sums was made by comparing  $R^2$  values between the fitted functions (Supplemental information Table S1). The models were fit using non-linear least-squares regression with the nls function (Baty et al., 2015) using R statistical software (Bates and Watts, 1988; R Development Core Team, 2013).



**Fig. 2.** Top panel: relationship between sampling date (the date of exposing the plants to forcing conditions) and the number of days required for the first bud to burst on each seedling (polynomial fitted functions;  $n = 1$  per sampling date per species). Black points show budburst requirements under the 8-h photoperiod, while white points show budburst requirements under the 16-h photoperiod. Bottom panel: chilling units which had accumulated up to each sampling date plotted against forcing units which had accumulated and led to budburst (exponential fitted functions for *F. sylvatica*, *A. alba*, *S. torminalis* and *L. decidua*, polynomial fitted functions for other species;  $n = 1$  per sampling date per species). Forcing units include the warm temperatures that occurred outside prior to each sampling interval, in addition to the forcing units required for bud-burst in the greenhouse. Small crosses represent the forcing unit sums which had accumulated outside at the field site only. Large crosses show the ambient bud burst dates of the tree seedlings under ambient conditions (no warming; top panel) and the respective forcing sums (bottom panel).



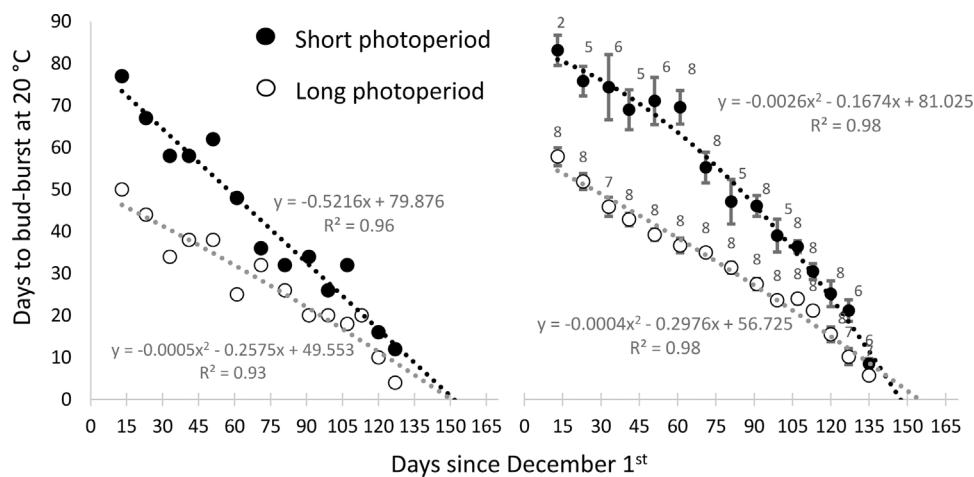


Fig. 3. Left panel: changes in budburst forcing requirements and photoperiod sensitivity in the provenance of *F. sylvatica* used in the among-species comparison (left panel,  $n=1$  per sampling point). Right panel: changes in budburst forcing requirements and photoperiod sensitivity pooled across all the eight longitudinally separated provenances of *F. sylvatica*. Numbers above the error bars show the sample size number of the provenances at each sampling date, for which days to budburst was observed in the climate chambers at 20 °C. Black and white points represent the budburst which occurred at the short photoperiod (8 h) and long photoperiod (16), respectively. Polynomial functions which best fitted the data are shown, with the respective coefficients of determination. Error bars denote standard error.

### 3.2. Photoperiod sensitivity of BFR

The photoperiod effect was tested using an ANCOVA:  $y \sim x * \text{photoperiod}$  (where  $y$  was days to budburst/forcing unit sum, and  $x$  was sampling date/chilling unit sum, respectively). An interaction between “ $x$ ” and photoperiod indicated that the photoperiod effect was dependent on the sampling date/chilling unit sum.

### 3.3. Explaining spring budburst with the fitted functions

Fitted sampling date vs. days to budburst functions were used to estimate the spring budburst date at the long photoperiod (16 h) and at the short photoperiod (8 h), with ambient budburst occurring when the photoperiod was between 16 and 8 h. Polynomial fitted functions were thus solved for the  $x$ -axis intercept (top panels of Fig. 2). For each species two values were obtained: the estimated budburst date at the long photoperiod and the estimated budburst date at the short photoperiod (using long and short photoperiod fitted functions, respectively). If the ambient spring budburst date (calendar day  $\pm$  SE) occurred between the two estimated budburst dates (at the long and short photoperiods), the spring budburst date was considered to have been projected accurately by the fitted functions (Table 3).

Fitted chilling unit sum vs. forcing unit sum functions were used to estimate the forcing unit sum required for budburst to occur under ambient conditions in the field. Spring budburst was considered to have been well explained when the actual accumulated forcing unit sum on the day of ambient budburst was within the range projected from the fitted long and short photoperiod functions. To estimate the required forcing unit sum ranges, the chilling unit sums up to the ambient spring budburst dates were input into the non-linear regressions (species-specific exponential or polynomial functions) at the long photoperiod and at the short photoperiod (bottom panels of Fig. 2; Table 3).

Spring budburst dates were estimated for the single *F. sylvatica* provenance used in the among-species comparison (Germany 3 provenance – Tables 1 and 2), as well as for the mean of all the eight *F. sylvatica* provenances (Fig. 3; Table 2). The correlations between sampling dates and forcing requirements, as well as the spring budburst dates, were very similar across all provenances, allowing for the pooling of the data (Fig. 3). The Germany 3 Provenance was chosen to represent *F. sylvatica* in the among species comparison because it had been grown under the same conditions as the other species, and its seed origin was most similar. For the provenances of *F. sylvatica*, an ANOVA was used to detect differences in spring budburst in the field. All statistical analyses were performed using R version 3.0.1 (R Development Core Team, 2013) and the additional package *sciplot* version 1.1-0. for advanced plotting. Homoscedasticity was verified using residual versus fitted plots, and the normality of residuals was tested with normal probability plots (Faraway 2005).

## 4. Results

### 4.1. Changes in BFR with respect to sampling date and chilling sum

At both the short and long photoperiods, days to budburst under the forcing conditions ( $+20$  °C), as well as the calculated forcing unit sums for the respective sampling dates, were negatively correlated with the sampling dates and the respective chilling unit sums (Fig. 2; Table 3). All regressions were significant ( $p < 0.05$ ), with a high goodness of fit (mean  $R^2 = 0.90 \pm 0.02$  SE for the fitted functions of sampling date vs. days to budburst, and mean  $R^2 = 0.82 \pm 0.04$  SE for the fitted functions of chilling unit sum vs. forcing unit sum). The constants for all of the polynomial and exponential functions are displayed in the Supplementary information (Table S1).

### 4.2. Photoperiod sensitivity changes from winter to spring

Overall, longer photoperiod shortened the days to budburst and forcing temperature sum requirements in *F. sylvatica*, *A. alba*, *T. cordata* and *S. tormalis* (Table 3: photoperiod effect). As expected, the effect of photoperiod decreased linearly with later sampling dates and accumulated chilling temperature sums for the three most photoperiod sensitive species (*F. sylvatica*, *A. alba* and *T. cordata*; Table 3: photoperiod interaction, Fig. 2). For *T. cordata* and *P. abies* the projected forcing unit requirements at budburst were counterintuitively higher at the short photoperiod than at the long photoperiod. For *P. abies* this is likely due to the higher data variability (much lower  $R^2$  compared to other species) and for *T. cordata* this is likely due to the clumping of points at the late sampling dates. Theoretically, the long and short photoperiod lines should converge at or after budburst, indicating that faster budburst at a shorter photoperiod at any point is impossible. The crossover of the two lines for the two mentioned species represents estimation error of the fitted functions.

### 4.3. Explanation of the budburst dates in the field

The fitted functions of sampling date vs. days to budburst estimated spring budburst dates in the field accurately for five of the eight species (Table 3–Projected budburst dates; top panels of Fig. 2). The fitted functions of chilling unit sum vs. forcing unit sum explained the forcing units required at ambient budburst accurately for all eight species (Table 3–Projected forcing unit sums; bottom panels of Fig. 2). At the time of budburst, 1 day equaled approximately 20 forcing units. If we convert the ranges of the estimated forcing units to days we get a mean range of 3 days, which is more precise than the mean precision from the fitted functions using sampling dates and days to budburst (mean range of 7 days).

Variation in days to budburst among the different provenances of *F. sylvatica* did not result in different projections of budburst dates (Fig. 3). The projected budburst period was 148–155 days (long and short photoperiod projections) since December 1 for the mean of the eight provenances, and 151–155 days for the “Germany 3” provenance used in the among-species comparison. There were also no significant differences among the eight ecotypes in the ambient spring budburst dates ( $p = 0.06$ ). All 37 *F. sylvatica* seedlings from the eight provenances exhibited bud burst within 4 days of each other. Likewise, the rate of loss of forcing requirements and photoperiod sensitivity were similar for all eight provenances (Fig. 3).

## 5. Discussion

Recent experimental evidence has shown that plant phenology is a complex phenomenon, and it is not simply driven by fall and spring temperatures. The documented reduction in the rate of advancement of spring green-up per degree of warming is a prime example of a non-linear response of plant phenology to climate warming (Fu et al., 2015). Historical phenological and climate records alone are thus insufficient to fully explain and project current and future phenological shifts. Predicting budburst dates under different temperature regimes will continue to be difficult until a more complete understanding of dormancy induction and release is acquired (Harrington et al., 2010). We demonstrated that the gradual release from dormancy in several common European tree species is simultaneously and continuously affected by the accumulated chilling temperature and photoperiod.

### 5.1. Temporal changes in budburst forcing requirements

As we hypothesized, the fitted functions correlating chilling unit sums and BFR accurately explained ambient spring bud burst dates in the field. Chilling, rather than photoperiod, has been hypothesized to play the primary role in climate change responses with respect to the onset of the growing season (Barr et al., 2004; Rollinson and Kaye, 2012). Therefore, when analyzing past changes in spring budburst phenology, changes in chilling and forcing units also should be considered, in addition to tracking changes in the calendar dates of budburst.

Contrary to our hypothesis, BFR patterns were not exponential from midwinter to spring for all species, as exemplified by *Q. robur* and *P. abies*, which exhibited non-exponential relationships at both long and short photoperiods (Fig. 2B). For the latter 2 species, we propose that BFR patterns may be sigmoidal (when followed back to the period of peak dormancy in late fall), maintaining a near constant BFR at the peak of dormancy, then increasing the rate of loss in BFC, before finally reaching the BFR asymptote. The BFR changes in *P. abies* and *Q. petraea* from winter to spring previously have been shown to be different from those of *F. sylvatica* by Basler and Körner (2014), although based on only three sampling dates, which makes a direct comparison with our study difficult. Species-specific rates of forcing accumulation at increasingly warm temperatures also have been observed (Caffarra and Donnelly, 2010). These results indicate therefore that the functions which relate temperature to chilling and forcing units (exponential vs. sigmoidal) may not be universal across species, as suggested previously by Harrington and Gould (2015). Using a single replicate per sampling date carries the cost of having reduced power to confirm the validity of the hump-shaped curves that we observed. Thus, future experiments should focus specifically on the fall and winter forcing requirements of these tree species to confirm our findings. It seems that if at an early enough sampling date (before dormancy is completely induced) the forcing requirements may be lower than during peak dormancy in midwinter. Future experiments should track dormancy from its onset to its reduction (i.e. from summer to spring) to test if the BFR patterns shown here can be reproduced. If they can, budburst prediction models which use exponential chilling unit sum vs. BFR functions may need to be

modified for each species, accordingly.

Laube et al. (2014) demonstrated that the ranking of species according to their budburst sensitivities to forcing temperatures can change from early to late winter. Also, species with high midwinter BFR do not necessarily burst later (Murray et al., 2014). Our results confirm and explain these findings. *F. sylvatica* had the highest peak BFR, yet its seedlings leafed out earlier or at the same time as *T. cordata* and *P. abies*. This response can be attributed to its faster rate of loss in BFR. *Larix decidua*, on the other hand, had a very low midwinter BFR from the start, despite having very gradual loss of BFR, and was therefore able to leaf out first in the spring. Thus, higher midwinter BFR delayed spring budburst, and a slower rate of decrease in BFR led to later spring budburst dates, which is consistent with our hypotheses. Both the maximum attained winter BFR and its rate of loss explain the changes in the rankings of species-specific forcing requirements with respect to the sampling date, as well as the final spring budburst.

### 5.2. Temporal changes in photoperiod sensitivity

Our results confirmed our hypothesis that photoperiod sensitivity is continuously negatively correlated with the accumulated chilling temperatures, which supplements previous findings (Caffarra and Donnelly, 2011; Laube et al., 2014b; Vitasse and Basler, 2013). The species that were the most photoperiod sensitive in our study (*F. sylvatica*, *A. alba* and *T. cordata*) also have been described as such in the literature (Basler and Körner, 2014; Laube et al., 2014b; Way and Montgomery, 2014; Zohner and Renner, 2015). For *P. abies*, however, increasing photoperiod during the forcing-conditions period has been shown to result in a significant photoperiod effect on BFC (Basler and Körner, 2014; Partanen et al., 1998). The forcing responses of *P. abies* were the most variable in our study (reflected in the lowest  $R^2$  for the fitted models), which could be a reason for the inaccuracy. Caution also needs to be exercised, given that the temperature sensitivities we observed were combined with a constant photoperiod being used during the forcing phases. However, naturally increasing photoperiod prior to the induced forcing conditions was recently found to have no effect on the photoperiod sensitivity and bud forcing requirements for at least three tree species (Zohner and Renner, 2015), including the species used here (*F. sylvatica*). Therefore, it appears that the accumulated chilling units, and not the progressive increase in the natural photoperiod, largely determine budburst photoperiod sensitivity.

### 5.3. Within-species differences

The well-described high chilling requirements of *F. sylvatica* (Heide, 1993a; Fu et al., 2015; Dantec et al., 2014) were confirmed in our study. Contrary to our hypothesis, however, the forcing requirements of *F. sylvatica* provenances from a broad longitudinal gradient reacted uniformly to chilling unit accumulation, as pooling the forcing requirements of all provenances resulted in similar regression lines between the sampling dates and the forcing requirements. Additionally, spring budburst dates did not differ among any of the *F. sylvatica* provenances. Previous studies have shown that a longitudinal cline exists for spring burst in *F. sylvatica*, with the earliest budburst occurring in eastern provenances (Von Wuehlich et al., 1995; Gömöry and Paule, 2011). There might be stronger differentiation among populations and provenances in the more southern range of *F. sylvatica*, where genetic variation in *F. sylvatica* is higher than in Central Europe. These patterns of genetic variation can be explained by the hypothesis that central Europe was colonized after the last ice age from only one refuge that contained small populations with a limited number of genotypes (Magri et al., 2006). Thus, our results suggest there is strong genetic control for chilling requirements in *F. sylvatica* (Gömöry and Paule, 2011; Chmura and Rozkowski, 2002) within similar latitudes.

As we hypothesized, photoperiod sensitivity was more similar within species (specifically, within *F. sylvatica*) than among species. All

of the provenances we examined stemmed from similar latitudes, and were thus accustomed to similar photoperiod regimes. Differences among species with respect to photoperiod sensitivity were present even though their latitudes of origin were similar. The increased variation in budburst for *Fagus sylvatica* under the short photoperiod was likely due to incomplete opening of the buds, especially during the beginning of the sampling period. Even though budburst did occur, leaf opening was at times incomplete or malformed. This result provides evidence that the chilling requirements were not met, and although an artificially extended photoperiod could fulfill them, such conditions are not encountered in nature at this time.

#### 5.4. Implications for species-specific budburst advancement

For *F. sylvatica*, the influence of photoperiod was much larger than for any other species, which may cause comparatively stronger limits on phenological advances for this species with warmer temperatures. As a consequence, *F. sylvatica* will potentially stop advancing its spring budburst phenology before other species in response to climate warming. Slow advances in spring budburst phenology in *F. sylvatica* have been reported previously (Aber et al., 2001; Heide, 1993a; Vitasse, 2013). In contrast, it has been suggested that *L. decidua* has an advantage over less photoperiod-sensitive species (typically late successional species) by being able to take advantage of warm winters (Körner and Basler 2010, Laube et al., 2014), which was supported by our results. The rest of the species we studied are likely to be intermediate in terms of their future potential spring budburst advancement. It has been suggested that early successional or pioneer species tend to exhibit low chilling and forcing requirements, and that they also have low photoperiod sensitivity (Basler and Körner, 2012; Körner and Basler, 2010; Laube et al., 2014). However, a recent literature review revealed that no clear characterization exists for classifying photoperiod sensitivity in trees (Way and Montgomery, 2014). Likewise, for the 8 tree species we examined, there were no attributes that were clearly associated with trends in the fitted functions. Additionally, whether the BFR and photoperiod sensitivity changes of adult trees are comparable to those of the seedlings we examined in our study remains to be determined.

In many temperate regions, the frequency and intensity of frost damage in the spring is increasing (Augspurger, 2013; Gu et al., 2008; Inouye, 2008). Species such as *L. decidua*, which stand to benefit from an earlier budburst (via an extended growing season), therefore need to be able to withstand sudden frost events after leaf out. *L. decidua* therefore needs to be frost hardened during budburst, and preliminary data suggest it has higher frost tolerance at leaf out compared to the other deciduous species in this study (personal observation). Vitasse et al. (2014) also have determined that species with early bud burst are the most frost tolerant as they leaf out. Therefore, species with low BFR may not be at a disadvantage when a sudden frost event occurs at leaf out, although frost occurrences after warming, but prior to budburst, need to be examined further.

## 6. Conclusions

In summary, tracking species-specific changes in BFR and photoperiod sensitivity at high temporal resolution with minimal replication explained differences in spring budburst dates and revealed novel, non-exponential, BFR patterns. Species with high BFR in mid-winter could, at least partially, offset the delay in spring budburst via faster rates of loss in BFR. Under climate change as the onset of spring warming advances and occurs when the buds are increasingly sensitive to photoperiod then bud burst will not advance as much as predicted by a forcing-only model.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agrformet.2017.09.011>.

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